[Jap. J. Parasit., Vol. 27, No. 6, 527-533, 1978]

Studies on the Plerocercoid Growth Factor of *Spirometra erinacei* (Rudolphi, 1819) with Special Reference to the Effect on Lipidmobilization *in Vitro*

KAZUMITSU HIRAI,† HIROSHI NISHIDA,† KUNINORI SHIWAKU† AND HIROMICHI OKUDA§

(Received for publication; August 15, 1978)

Introducton

Plerocercoid Growth Factor (PGF) was first demonstrated by Mueller (1963), who showed a growth stimulating effect in mice infected with plerocercoids of *Spirometra mansonoides* (Mueller, 1935). He also reported that the mice infected with oriental strain of *Spirometra* collected from Malay, Taiwan and Australia showed less increased weight gain (about half as much) comparing with *S. mansonoides*, but oriental strain had little or no effect on hypophysectomized and propylthiouracil-treated rats (Mueller, 1968; 1970). It was found that PGF was produced exclusively from plerocercoids of *S. mansonoides*, and could accelarate the weight gain in several laboratory animals, both intact (Mueller, 1965; Phares and Ruegamer, 1973) and hormonally deficient animals such as thyroidectomized, hypophysectomized (Mueller, 1968) or alloxan diabetic rats (Ruegamer and Mueller, 1973). As to the relation between the plerocercoid and its host in lipid metabolism, Meyer *et al.* (1965) reported that the weight gain in plerocercoid-infected hamsters was due to increased fat. They also demonstrated that plerocercoids of *S. mansonoides* lacked the mechanisms required for the synthesis de novo of its sterol and long chain fatty acids, and possessed the mechanism for the synthesis of its own triglycerides, sterolesters and phospholipids with the use of exogenously supplied sterols and fatty acids (Meyer *et al.*, 1966).

Harlow *et al.* (1967) described that an extract of plerocercoids possessed an insulin-like activity such as stimulating glucose oxidation and utilizing glucose for lipogenesis in epididymal fat pads of rats.

On the other hand, Steelman *et al.* (1971) reported that PGF had functional similarity to growth hormone except for lipid metabolism. Phares and Carroll (1977) found that PGF exhibited such a lipogenic effect in plerocercoid-infected hamsters as increasing epididymal fat pads weight, serum triglycerides, cholesterol, total lipids and stimulating incorporation of [2-^{14}C] acetate into the liver and serum.

In the light of these reports, the present investigation demonstrates the existence of PGF in the plerocercoid of *Spirometra erinacei* (Rudolphi, 1819) in Japan and characterization of the PGF.

Materials and Methods

Animals: Female ICR mice, weighing approximately 10 g, were purchased from

† *Department of Parasitology, School of Medicine, Ehime Univesity, Ehime 791-02, Japan.*
§ *2nd Department of Medical Biochemistry, School of Medicine, Ehime Universiy, Ehime 791-02, Japan.*

Japan Animal Co. (Osaka, Japan).

Plerocercoids: Plerocercoids of *S. erinacei* were collected from two species of snakes, *Elaphe quadrivirgata* and *Rabdophis tigrinus*, captured in southern area of Ehime Prefecture, Japan.

Reagents: Collagenase, propylthiouracil and pronase were purchased from Sigma Chemical Co., bovine plasm albumine powder from Armour Pharmaceutical Co., and penicillin-G from Meiji Co.

Preparation of the incubation medium of plerocercoids, the worm homogenate and the extract of the worm: Plerocercoids collected from snakes were rinsed in physiological saline solution containing 1,000 u/ml penicillin-G, and the same solution was added to the rinsed plerocercoids at the rate of 25 plerocercoids per 10 ml of the solution. Thereafter, the plerocercoids were incubated at 30 C for 12 hr. At the end of incubation period, supernatant was decanted and subjected to centrifugation at 3,000 rpm for 10 min. The supernatant solution was dialysed against distilled water for 48 hr. All operations were carried out at 4 C following the incubation. The supernatant dialysed was lyophilized and resultant powder was then dissolved in 2.0 ml of distilled water. The solution was used as incubation medium. The worm homogenate and its extract were prepared by the following procedure: about 200 plerocercoids collected from snakes were washed in distilled water, and placed in 10 ml of Krebs-Ringer phosphate buffer at pH 7.4, and then ground with a Teflon homogenizer. After the homogenized plerocercoids was centrifuged at 3,000 rpm for 10 min, the supernatant fluid was used as extract of the worm.

Infection of mice: Twenty female ICR mice weighing about 10 g were randomly sorted into 4 groups of 5 mice each, of which 2 groups were for the experimental and the remaining two for the control group. All the mice were ear-coded, and maintained on shavings in cages, with pelleted mouse food and water *ad lib*. Each mouse of two experimental groups was injected under the dorsal skin with 9 scoleces in 0.5 ml of physiological saline solution containing 1,000 u/ml penicillin-G. The controls received an equivalent injection of penicillin-G without worms. After receiving the injection, each mouse of experimental and control groups was provided with propylthiouracil (PTU) in the drinking water in the proportion of 200 mg per liter. Animals of each group were weighed individually once a week for 10 weeks, and average weight gain was plotted on the graph to draw the growth curve of each group.

In vitro assay of lipid metabolism: The suspended solution of fat cells isolated from the epididymal adipose tissue of Wistar strain rats, weighing approximatelly 200–250 g, was prepared by the method of Rodbell (1964). The reaction mixture containing 0.5 ml of suspended solution of fat cells, 0.1 ml of either the incubation medium, the worm homogenate or the worm extract, 1.0 μg of adrenaline and 0.5 ml of Krebs-Ringer phosphate buffer (pH 7.4) containing 5% bovine plasma albumine was incubated at 37 C for 120 min. But on the assay used bovine growth hormone, Krebs-Ringer phosphate buffer was replaced by Krebs-Ringer bicarbonate buffer (pH 7.4). The reaction was terminated by addition of Dole's extractions mixture. Free fatty acid (FFA) was assayed by the method of Dole (1951). The released FFA values were corrected for FFA content at 0 time. On the other hand, the controls without adrenaline (or bovine growth hormone) or the sample were also prepared and assayed by the same method, and the released FFA values were compared with FFA content of the control.

Protein concentration in samples was determined by the method of Lowry *et al.* (1951).

Results

Experiment on non-treated mice:

In female ICR mice receiving 9 scoleces with an average starting weight of 10.9 g,

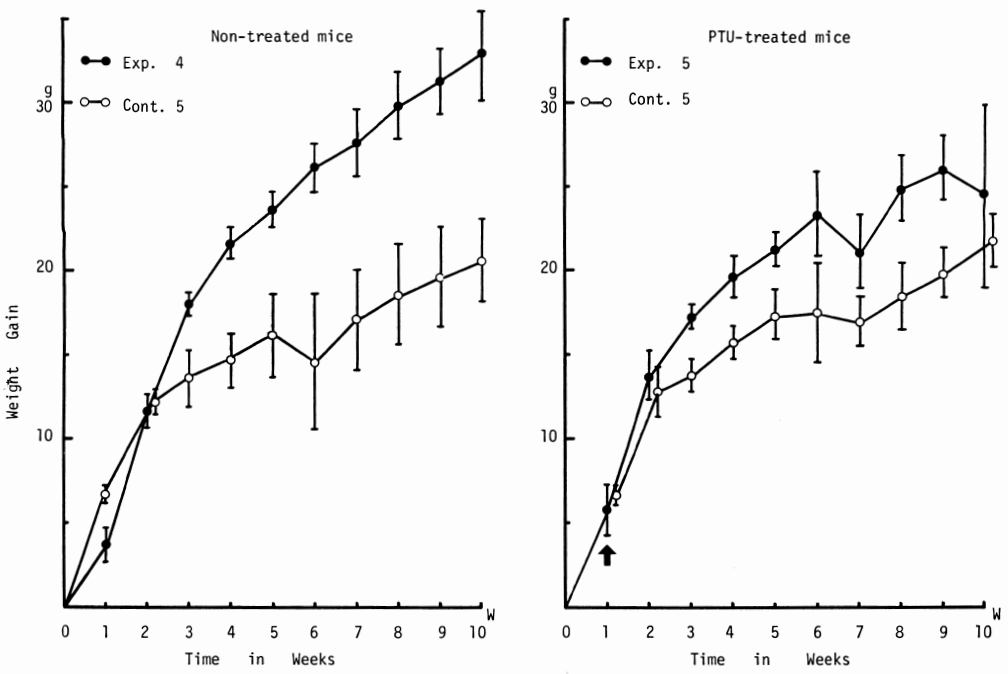

Fig. 1 Growth curves of non-treated and PTU-treated female mice with or without plerocercoids. Experimental mice were injected under the dorsal skin with 9 scoleces of *S. erinacei* plerocercoid. The controls were injected with blank injection. Solid circles represent the experimentals, open circles the controls. Upright lines represent standard deviation.

and those receiving no scoleces with an average starting weight of 11.2 g, the weight gain of the experimentals lagged bihind that of the controls for the first two weeks, and one of these mice died of unknown causes in this period. At the end of the 3rd week, however, the experimentals significantly showed the weight gain over the controls ($P<0.05$), and thereafter the greater gain was usually kept through the rest of the experimental period ($P<0.005$).

The experiment was terminated at 10 weeks, at which time the experimentals were weighed 44.2 g, vs. 31.9 g for the controls, and an average weight gain of the experimentals was 1.6 times as much as the controls (Fig. 1).

Experiment on propylthiouracil-treated mice :

At the end of the first week, each of the experimentals received 9 scoleces and the controls received sham injection without plerocercoids, both were kept on propylthiouracl (PTU) in the drinking water thereafter. The weight gain of the experimentals was evidently greater than that of the controls, in spite of the PTU treatment ($P<0.05$).

Although one of the experimentals decreased the weight at the 10th week, the average weight gain of the experimentals reached 36.9 g, vs. 30.9 g for the controls at the end of 9 weeks. The advantage of the weight gain on the PTU-treated experimentals was obviously a little greater than that of the non-treated experimentals (Fig. 1), but the PTU-treated experimentals was growing greater than the controls of non-treated mice ($P<0.05$).

In vitro experiment :

An effect of the incubation medium on adrenaline-induced lipolysis was examined using isolated fat cells. The results are shown in Fig. 2. Addition of the incubation medium increased lipolysis both in the

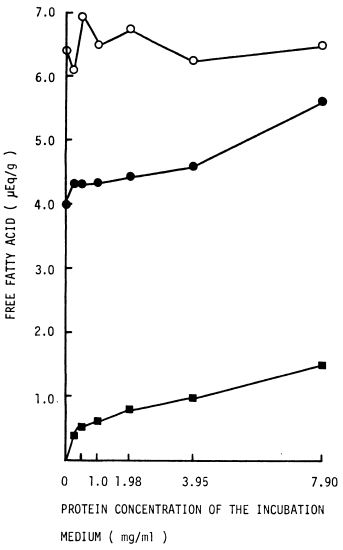

Fig. 2 Effect of the incubation medium on adrenaline-induced lipolysis in fat cells. Solid circles represent the unboiled incubation medium, open circles the boiled incubation medium, and square the absence of adrenaline.

presence and absence of adrenaline. When the incubation medium was previously boiled in the water bath for 5 min., lipolytic activity of the incubation medium was disappeared. On the other hand, the worm homogenate and its extract inhibited adrenaline-induced and bovine growth hormone-induced lipolysis in isolated fat cells

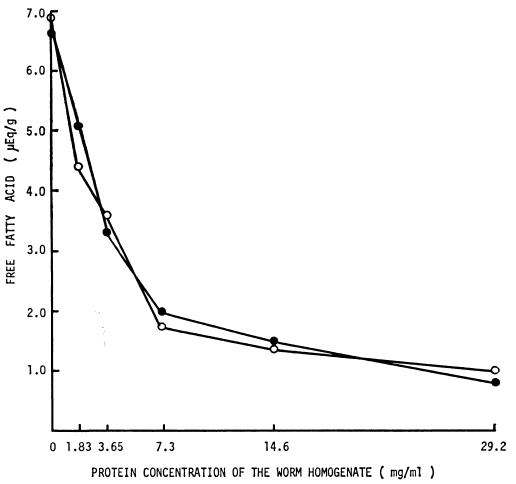

Fig. 3 Effect of the worm homogenate on adrenaline-induced lipolysis in fat cells. Solid circles represent the unboiled worm homogenate and open circles the boiled worm homogenate.

(Figs. 3, 4). In contrast to the incubation medium, the worm homogenate and the extract of the worm homogenate retained the inhibitory activity of adrenaline-induced lipolysis after boiling for 5 min. (Figs. 3, 4). The worm homogenate was incubated with pronase, followed by boiling for 5 min. The resultant heat-treated mixture was subjected to estimation of inhibitory activity of adrenaline-induced lipolysis. It was found that the pronase treatment decreased the inhibitory activity. This fact

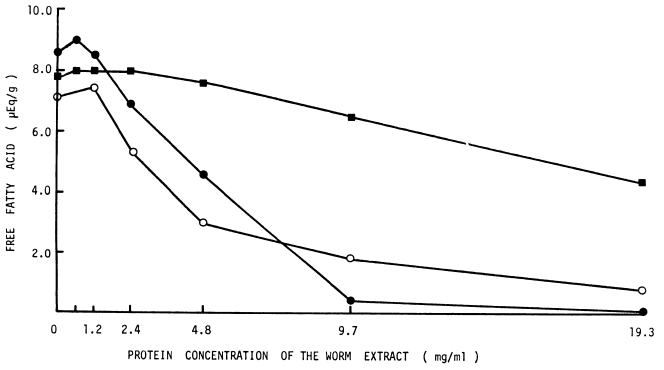

Fig. 4 Effect of the extract from the worm homogenate on adrenaline- and bovine growth hormone-induced lipolysis in fat cells. Solid circles represent effect of the untreated extract, open circles effect of the boiled extract, and square represent effect of the untreated extract on bovine growth hormone-induced lipolysis.

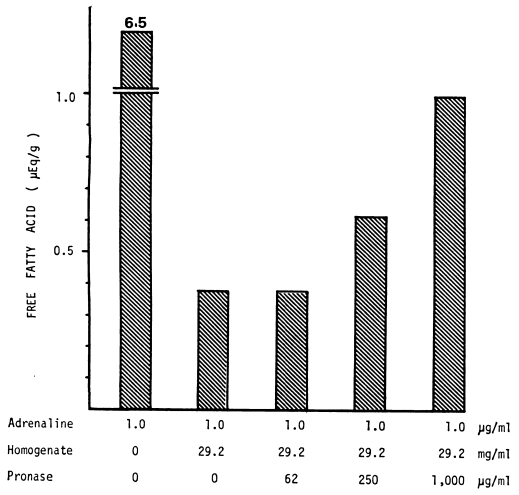

Fig. 5 Effect of the worm homogenate treated with pronase on adrenaline-induced lipolysis in fat cells.

suggested that an active substance of the worm homogenate might be proteinaceous in nature (Fig. 5).

Discussion

It was clarified that the plerocercoids of *Spirometra erinacei* (Rudolphi, 1918) in Japan showed a growth stimulating effect on non-treated and PTU-treated mice. Non-treated female ICR mice receiving 9 scoleces subcutaneously showed increase of the body weight as much as that produced by *S. mansonoides* (Mueller, 1963). Mice also showed increase of the body weight by the treatment with PTU as shown in rats by Mueller and Reed (1968).

These results suggested that the plerocercoid of *S. erinacei* in Japan may produce PGF in mice. The present investigation clarified that the incubation medium of plerocercoids promoted lipolysis in isolated fat cells, but worm homogenate and the extract inhibited it *in vitro*.

These facts suggested that the biological activity of our incubation medium was similar to growth hormone-like activity confirmed in the incubation medium by Phares and Ruegamer (1973), and that the activity of the worm homogenate and its extract corresponded to insulin-like activity as shown by Harlow *et al.* (1967). However, Steelman *et al.* (1971) reported that PGF did not enhance lipolysis and did not produce the elevation of serum lipid levels *in vivo*. Phares and Carroll (1977), on the other hand, recently reported that PGF exhibited the lipogenic effect *in vivo*. In our experiment, it seems that there exist two factors, growth hormone-like and insulin-like factor in the plerocercoid of *S. erinacei* in Japan. The growth hormone-like factor of the incubation medium was labile to heat-treatment, and not dialysable. The insulin-like factor of the worm homogenate and its extract was obviously stable to heat-treatment, and inactivated by pronase treatment. These results suggest that the insulin-like factor is proteinaceous in nature.

Summary

This report demonstrated that the incubation medium of plerocercoids had lipolytic activity, and that the worm homogenate and its extract significantly inhibited adrenaline- and bovine growth hormone-induced lipolysis in isolated epididymal fat cells *in vitro*. In addition, it was confirmed that the plerocercoid of *Spirometra erinacei* (Rudolphi, 1819) from the snakes in Japan, also produced a growth stimulating substance in non-treated and PTU-treated mice as much as that of *S. mansonoides*. While the lipolytic factor in the incubation medium was heat-labile, the antilipolytic factor in the worm homogenate and its extract was found to be heat-stable and easily inactivated by the treatment of pronase.

Acknowledgments

We thank Miss Etsuko Takeda for technical assistances and the help of Mr. Masahiro Sakai is gratefully acknowledged.

References

1) Dole, V. R. (1951): A relation between non-esterified fatty acids in plasma and the

metablism of glucose. J. Clin. Invest., 35, 150–154.
2) Harlow, D. R., Mertz, W. and Mueller, J. F. (1967): Insulin-like activity from the sparganum of *Spirometra mansonoides*. J. Parasit., 53, 449–454.
3) Lowry, O. H., Rosebrough, N. J., Farr, L. A. and Randall, R. J. (1951): Protein measurement with the forin phenol reagent. J. Biol. Chem., 193, 265–275.
4) Meyer, F., Kimura, S. and Mueller, J. F. (1965): Stimulation of lipogenesis in hamsters by *Spirometra mansonoides*. J. Parasit., 51 (Suppl.), 143.
5) Meyer, F., Kimura, S. and Mueller, J. F. (1966): Lipid metebolism in the larval and adult forms of the tapeworm *Spirometra mansonoides*. J. Bio. Chem., 241, 4224–4232.
6) Mueller, J. F. (1963): Parasite-induced weight gain in mice. Ann. N. Y. Aca. Sci., 133, 216–233.
7) Mueller, J. F. (1965): Further studies on parasitic obesity in mice, deer mice, and hamsters. J. Parasit., 51, 523–532.
8) Mueller, J. F. and Reed, P. (1968): Growth stimulation induced by infection with *Spirometra mansonoides* spargana in propylthiouracil-treated rats. J. Parasit., 51, 51–54.
9) Mueller, J. F. (1968): Growth stimulating effect of experimental sparganosis in thyroidectomized and hypophysectomized rats, and comparative activity of different species of *Spirometra*. J. Parasit., 54, 795–801.
10) Mueller, J. F. (1970): Comparision of the growth-promoting effect of *Spirometa mansonoides* vs. three oriental forms in intact mice and hypophysectomized rats. J. Parasit., 56, 842–844.
11) Mueller, J. F. (1974): The biology of Spirometra. J. Parasit., 60, 3–14.
12) Phares, C. K. and Ruegamer, W. R. (1973): In vitro preparation of a growth factor from plerocercoids of the tapeworm, *Spirometra mansonoides*. Proc. Soc. Exp. Biol. Med., 142, 374–377.
13) Phares, C. K. and Carroll, R. M. (1977): A lipogenic effect in intact male hamsters infected with plerocercoids of the tapeworm, *Spirometra mansonoides*. J. Parasit., 63, 690–693.
14) Rodbell. M. (1964): Metabolism of isolated fat cells. J. Bio. Chem., 239, 375–380.
15) Ruegamer, W. R. and Mueller, J. F. (1973): Growth responses in thyroidectomized and alloxan diabetic rats infected with plerocercoids of the tapeworm, *Spirometra mansonoides*. J. Nutr., 103, 1496–1501.
16) Steelman, S. L., Glitzer, M. S., Ostrid, D. A. and Mueller, J. F. (1971): Biological properties of the growth hormonelike factor from the plerocercoids of *Spirometra mansonoides*. Recent Prog. Horm. Res., 27, 97–120.

マンソン裂頭条虫 *Spirometra erinacei* (Rudolphi, 1819) の擬充尾虫由来の Plerocercoid Growth Factor についての研究，特に *in Virto* における脂肪動員作用に対する影響

平井和光　西田　弘　塩飽邦憲
(愛媛大学医学部寄生虫学教室)

奥　田　拓　道
(愛媛大学医学部第Ⅱ生化学教室)

愛媛県にて捕獲されたシマヘビ，ヤマカガシより採取されたマンソン裂頭条虫 *Spirometra erinacei* の擬充尾虫の頭節を健康な ICR 系マウスの背部皮下に9頭節接種した結果，マウスは，生理的食塩水のみを接種された対照群に比較して有意に ($P<0.005$) 成長が促進された．

また，プロピールチオウラシル含有飲料水 (200mg/l) を摂取しているマウスに対しても同様に成長促進を認めた ($P<0.05$)．

一方，この擬充尾虫を12時間培養した生理的食塩水を透析後，真空凍結乾燥して濃縮した培養液中に Adrenaline-induced Lipolysis を促進する生物学的活性物質を認めた．この物質は，熱処理により活性を喪失した．また，擬充尾虫体のホモジネート及びその上清中には，Adrenaline または Bovine growth hormone-induced Lipolysis を抑制する生物学的活性物質を認めた．これは，熱処理により活性を喪失せず，プロナーゼ処理により活性を喪失した．即ち，マンソン裂頭条虫の擬充尾虫は，*Spirometra mansonoides* のそれと同様に Plerocercoid Growth Factor を有し，その生物学的活性は，培養液中のものには，脂肪動員作用があり，虫体中には，脂肪動員抑制作用を持っていることを認めた．そして，この活性物質は，蛋白質であることが推測された．